Laboratory measurements of immersion freezing abilities of non-proteinaceous and proteinaceous biological particulate proxies

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Abstract

Non-proteinaceous and proteinaceous biological aerosols are abundant within the atmosphere and have the potential to impact the climate through cloud and precipitation formation. In this study, we present the differences in the laboratory-measured freezing capabilities of the non-proteinaceous and proteinaceous biological materials to determine which has more potential to impact the ice nucleation in the clouds. As non-proteinaceous surrogates, we examined multiple cellulose materials (e.g., microcrystalline and nanocrystalline cellulose) whose sizes range from ˜100 nm to >100 μm (according to manufacturer report). For proteinaceous proxies, we looked at different gram-negative bacteria, such as Pseudamonas aeruginosa, Escherichia coli, Serratia marcescens, Citrobacter freundii, and Snomax, (which contains P. syringae) that can be found around the proximity of the Texas Panhandle. By using the Cryogenic Refrigeration Applied Freezing Test (CRAFT) system, we estimated immersion freezing efficiency (i.e., ice nucleation activity scaled to a unit of mass) of each sample at the temperatures greater than -30°C. We have observed that not all gram-negative bacteria has high immersion freezing activity, but the few do have a warmer temperature onset (>-20 °C) than the cellulose used. For those that did not exhibit substantial freezing efficiencies, they had similar freezing properties as the broth, in which the bacteria were incubated, as well as the cellulose materials examined. These observations suggest the presence and potential importance of bacterial cellulose in the atmospheric ice nucleation. From here, we need to conduct more in-depth investigation in the effects of a wider variety of atmospherically relevant biological aerosols to get a better understanding of the effects of said aerosols on overall aerosol-cloud interactions. Acknowledgments: K. Cory would like to acknowledge NSF-EAPSI and JSPS Summer Program for the travel fellowship support. N. Hiranuma acknowledges financial aids by the Higher Education Assistance Fund (HEAF), WTAMU Office of Graduate School and Killgore Research Center.

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Overview

Glaciation of atmospheric clouds by immersion freezing is an important atmospheric process, which is affecting the formation of cloud and precipitation, as well as Earth's energy budget (Boucher et al., 2013). Our research using surrogates of biological ice-nucleating particles is important in the Texas Panhandle where both non-proteinaceous and proteinaceous biological aerosols are abundant in the atmosphere. This study presents the differences in the laboratory-measured freezing capabilities of the biological aerosols to determine which has more potential to impact the ice nucleation in the clouds. Our research outputs will be valuable for predicting regional impact of aerosol-cloud interactions in the Southern Great Plains.

Biological Samples

ID	Sample	Origin	wt%	droplet size	Location of Experiment
1	Microcrystalline Cellulose (MCC)	Aldrich, 435236	0.05-0.005	5µL	WTAMU
2	Fibrous Cellulose (FC)	Sigma, C6288	0.05-0.005	5µL	WTAMU
3	Nanocrystalline Cellulose (NCC)	Melodea, NCC (3 wt%)	0.1-0.0001	5µL	NiPR
4	Cellulose Nano Fiber (CNF)	Nippon Paper Industries (NPI), TEMPO-CNF(short, 1.0 wt%)	0.1-0.00001	5µL	NiPR
5		NPI, TEMPO-CNF(standard, 1.0 wt%)	0.1-0.0001	5µL	NiPR
6		NPI, CM-CNF (1.1 wt%)	0.1-0.00001	5µL	NiPR
7		NPI, CM-CNF (powder, 94.8 wt%)	0.1-0.01	5µL/3µL	NiPR/WTAMU
8	*P. aeruginosa*	Ward's, VWR 470179-540	0.1	3µL	WTAMU
9	Snomax (*P. syringae*)	York International (Polen et al., 2016)	0.1	5µL	WTAMU
10	*E. coli* (K-12)	Ward's, VWR 470179-508	0.5-0.05	5µL	WTAMU
11	*S. marcescens*	Ward's, VWR 470176-540	0.5-0.05	5µL	WTAMU
12	*C. freundii*	Ward's, VWR 470179-496	0.5-0.05	5µL	WTAMU

- Non-proteinaceous particles (ID 1-7) are abundant in the atmosphere with a biannually measured mass concentration of >0.1 µg m^{-3} (Hiranuma et al., 2015).
- Proteinaceous biological particles (ID 8-12) may consist of gram-negative bacteria that may contain ice nucleation active proteins on the outer membrane (Silhavey et al., 2010).

Bacterial Culturing Procedure

1) Bacterium was T-streaked onto an agar plate and incubated for 24 hours.
2) One colony is suspended into a 1mL of aqueous broth and, then, incubated for 24, 48, and 72 hours.
3) Incubated vials are then centrifuged for 15 minutes at 13,000 rotations per minute and additional two rounds of 5 minutes at 13,000 rotations per minute to remove the supernatant and generate the bacteria "pellet".
4) Once all the supernatant was removed, we weighed the individual pellet to ~10mg to create a .1wt% solution with autoclaved Milli-Q water.

Fig. 1. T-streak of *C. freundii* on a Trypticase Soy Agar (TSA) plate

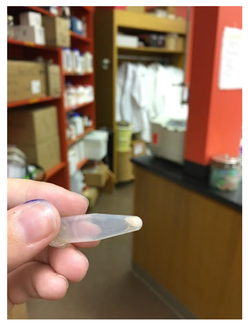

Fig. 2. "Pellet" of *P. aeruginosa* in a 1.5mL tube prior to weighing

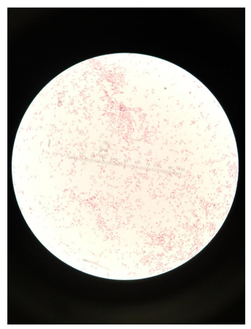

Fig. 3. Gram stain of *E. coli* under microscopy at 1000x magnitude

Ice Nucleation Experiment

Immersion freezing experiments were performed using two cold stage droplet assay platforms:

1) Using the Cryogenic Refrigerator Applied Freezing Test system (CRAFT) (Tobo, 2016) located at the National Institute of Polar Research (NiPR) in Tachikawa, Japan, 49 solution droplets (5 µL each) placed on a Vaseline layer were analyzed for each experiment.
2) Using the WT-CRAFT replicated at West Texas A&M University (WTAMU) in Canyon, TX, 70 solution droplets (3 µL each) placed on a Vaseline layer were analyzed per experiment.

The freezing ability of super-microliter-sized droplets (3-5 µL) containing our biological samples at a set weight percent were examined at a cooling rate of 1 °C min^{-1}. Frozen fraction and ice nucleation active mass density (n_m) were estimated as a function of temperature for every 0.5°C based on the phase transition visually observed in each system.

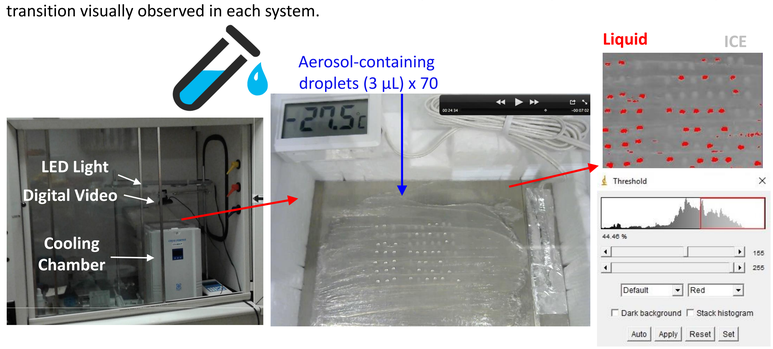

Fig. 4. West Texas Cryogenic Refrigerator Applied to Freezing Test (WT-CRAFT) system: simulation of atmospheric ice nucleation using aerosol-containing supercooled droplets. Ice nucleation is determined optically based on the change in droplet brightness when the initially transparent liquid droplets become opaque upon freezing. If the freezing temperature is not obvious for any droplets, the 8-bit grayscale images can be assessed on the ImageJ software to determine the temperature of phase shift for suspicious droplets by varying the minimum threshold gray value of 155-175 at the fixed maximum threshold value of 255.

Preliminary Results: Frozen Fraction

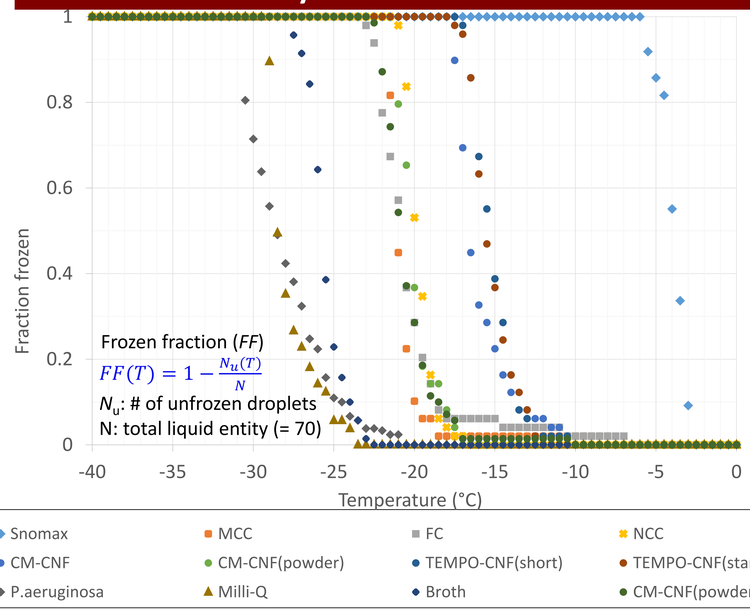

Fig. 5. Fraction of frozen droplets as a function of temperature. All solutions were conducted at a 0.1wt% except MCC/FC (0.05wt%). Broth and Milli-Q are pure concentrations with no added components. **We have examined all other proteinaceous samples, but the preliminary results showed no difference from the broth.**

Preliminary Results: n_m

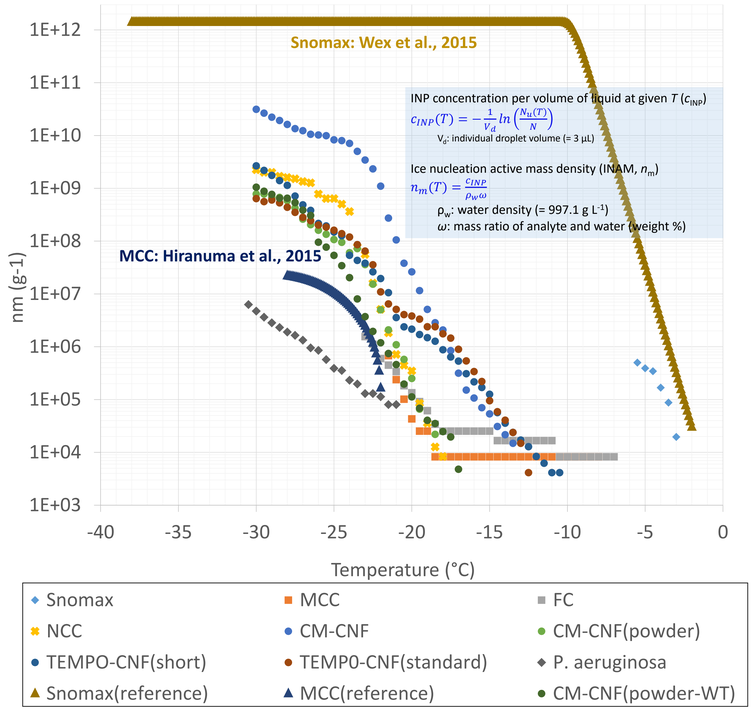

Fig. 6. Ice nucleation active mass density per unit mass for all solutions as a function of temperature (T).

Summary

- Not all gram-negative bacteria are ice nucleation active.
- Cellulose is in general more active when compared to our cultured bacteria.
- Different cellulose materials exhibit various ice nucleation abilities (3 orders magnitude difference in n_m at -20 °C).
- NCC shows a similar ice nucleation spectrum as the CM-CNF (powder).

Outlook

- We need to conduct a more in-depth investigation in the effects of a wider variety of atmospherically relevant biological aerosols to get a better understanding of the effects of said aerosols.
- Another step we need to take is focusing primarily on the warmer temperatures (-5°C to -15°C) with a reduced amount of particles and reduced size of droplets.
- We would need to focus on a reduced weight percent to simulate a condition of single particle immersed in each droplet.
- Investigating the ambient transport of bio-aerosols from mid- to high-latitude is necessary to develop future composition-resolved weather and climate models.

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